PB82-181735

International Study of 'Artemia' IX. Lipid Level Energy Content and Fatty Acid Composition of the Cysts and Newly Hatched Nauplii from Five Geographical Strains of 'Artemia'

Rhode Island Univ.
Kingston

Prepared for

Environmental Research Lab.
Narragansett, RI

1980

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/D-82-223	2. Book Chapter
3. REC PB82-181785	
4. TITLE AND SUBTITLE International Study of Artemia IX. Lipid Level, Energy Content and Fatty Acid Composition of the Cysts and Newly Hatched Nauplii From Five Geographical Strains of Artemia	5. REPORT DATE March 1982 1980
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) P.S. Schauer(1), D.M.Johns(2), C.E. Olney(1), and K.L.Simpson(1)	8. PERFORMING ORGANIZATION REPORT NO. ERLN-B265
9. PERFORMING ORGANIZATION NAME AND ADDRESS (1) Dept of Food Science and Technol., Nutrition and Dietetics, Univ of RI, Kingston, RI,02881 and (2) U.S.EPA, ERL, Narragansett, S. Ferry Rd, Narragansett, RI	10. PROGRAM ELEMENT NO. ACUL1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S.EPA, ERL, Narragansett South Ferry Road Narragansett, RI, 02882	13. TYPE OF REPORT AND PERIOD COVERED Book Chapter
	14. SPONSORING AGENCY CODE EPA/600/05

15. SUPPLEMENTARY NOTES IN: The Brine Shrimp Artemia.1980.Vol.3.Ecology,Culturing, Use in Aquaculture. G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds.) Universa Press, Wetteren, Belgium. pp. 365-373

16. ABSTRACT

Artemia cysts and newly hatched nauplii from Australia, Brazil, Italy and the United States (California and Utah) were analyzed for their total lipid level, total fatty acid level and composition, and their energy content in an effort to evaluate their lipid nutritional value as diets of marine organisms. Results are compared to biological data from a nutritional evaluation of these five *Artemia* strains on various marine organisms.

The total lipid, fatty acid methyl ester and energy levels of all strains appeared to be adequate to promote good growth and survival of the marine organisms. The fatty acid spectrum of the cysts and nauplii were nearly identical, indicating that the cyst shell contains little fatty acid-type lipids. However, significant differences were found in the fatty acid composition between the various strains.

Artemia were classified into two groups based on their $\omega 3$ polyunsaturated fatty acid composition. The major difference between the two groups was that one group contained predominantly 18:3ω3 and 18:4ω3, while the other group contained chiefly 20:5ω3. Considering the importance of 20:5ω3 to marine organism nutrition, the Australia, Italy, Brazil, and San Francisco Bay 321 strains which contained the higher level of this fatty acid would provide the best nutrition of the five strains. The San Pablo Bay 1628, San Francisco Bay 313 and Utah strains would probably be of less nutritional value due to the low level of 20:5ω3 and/or the excess amount of 18:3ω3. It is possible, however, that there is an interaction between an essential fatty acid (20:5ω3) deficiency and a dietary contaminant. This possibility is discussed with reference to biological results obtained when these five *Artemia* strains were fed to three different marine organisms.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT Distribution Unlimited	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

EPA-600/D-82-223
Book Chapter

The Brine Shrimp Artemia. *1980. Vol. 3. Ecology, Culturing, Use in Aquaculture*
G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

International Study on *Artemia*[1] IX. Lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from five geographical strains of *Artemia*

Paul S. Schauer[2], *D. Michael Johns*[3], *Charles E. Olney*[2], *and Kenneth L. Simpson*[2]

[2] *Department of Food Science and Technology, Nutrition and Dietetics, University of Rhode Island Kingston, Rhode Island 02881, USA*

[3] *Environmental Research Laboratory, United States Environmental Protection Agency South Ferry Road, Narragansett, Rhode Island 02882, USA*

Abstract

Artemia cysts and newly hatched nauplii from Australia, Brazil, Italy and the United States (California and Utah) were analyzed for their total lipid level, total fatty acid level and composition, and their energy content in an effort to evaluate their lipid nutritional value as diets of marine organisms. Results are compared to biological data from a nutritional evaluation of these five *Artemia* strains on various marine organisms.

The total lipid, fatty acid methyl ester and energy levels of all strains appeared to be adequate to promote good growth and survival of the marine organisms. The fatty acid spectrum of the cysts and nauplii were nearly identical, indicating that the cyst shell contains little fatty acid-type lipids. However, significant differences were found in the fatty acid composition between the various strains.

Artemia were classified into two groups based on their $\omega 3$ polyunsaturated fatty acid composition. The major difference between the two groups was that one group contained predominantly 18:3ω3 and 18:4ω3, while the other group contained chiefly 20:5ω3. Considering the importance of 20:5ω3 to marine organism nutrition, the Australia, Italy, Brazil, and San Francisco Bay 321 strains which contained the higher level of this fatty acid would provide the best nutrition of the five strains. The San Pablo Bay 1628, San Francisco Bay 313 and Utah strains would probably be of less nutritional value due to the low level of 20:5ω3 and/or the excess amount of 18:3ω3. It is possible, however, that there is an interaction between an essential fatty acid (20:5ω3) deficiency and a dietary contaminant. This possibility is discussed with reference to biological results obtained when these five *Artemia* strains were fed to three different marine organisms.

Introduction

Artemia (brine shrimp) is a widely used food source in laboratory and commercial rearing of many marine organisms. Several new geographical *Artemia* strains have recently become

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

available and a subsequent need has developed for their analysis as potential supplements or replacements for the present commercial sources.

An important determinant of the overall nutritional value of any food stuff is its lipid content. Triglyceride-type lipids are a major source of a diet's metabolizable energy and are directly linked to the growth of the consumer organism (Pandian, 1975). The dietary fatty acid composition ultimately determines the fatty acid composition of the structural phospholipids (Castell *et al.*, 1972ab ; Norred and Wade, 1972). Phospholipids are functionally active in maintaining proper membrane fluidity and cellular transport mechanisms. Recent research has demonstrated that the $\omega 3$ polyunsaturated fatty acids (PUFA) are required for lobsters (Castell and Covey, 1976), prawns (Guary *et al.*, 1976 ; Kanazawa *et al.*, 1979) and for several marine fish, including plaice (Owen *et al.*, 1972), red sea bream (Yone and Fujii, 1975a) and turbot (Cowey *et al.*, 1976).

The purpose of this research was to determine the total lipid content, fatty acid composition and energy content of cysts and newly-hatched nauplii of *Artemia* from five geographical regions.

Materials and methods

Sources and culturing of *Artemia*

Dehydrated cysts from five geographical locations were provided by the Artemia Reference Center (Ghent, Belgium). The origin of these cysts were Shark Bay, Australia (World Ocean, lot no. 113) ; Macau, Brazil (Companhia Industrial do Rio Grande do Norte, CIRNE-Brand, harvested 1978) ; Margherita di Savoia, Italy (harvested 1977) ; Great Salt Lake, Utah, USA (harvested 1977) ; San Pablo Bay, California, USA (Living World, San Francisco Bay Brand, Inc., lot no. 1628) and two samples from San Francisco Bay, California, USA (San Francisco Bay Brand, Inc., lot no. 313/3006 and lot no. 321995). The latter three Californian samples will be referred to by their respective full or abbreviated lot numbers : SP 1628, SF 313 and SF 321.

Stage one *Artemia* nauplii were hatched from cysts incubated at 25 °C in 30 ‰ filtered (0.45 μm) seawater for a specific period of time that was dependent on the particular strain (Johns *et al.*, 1980).

Samples were either held at -20 °C until lipid analysis or dried at 60 °C to a constant weight for determination of the energy content.

Lipid extraction and analysis

Total lipids

Cysts were ground in a mortar and pestle and extracted twice with chloroform/methanol/water (20 ml/40 ml/16 ml) (Bligh and Dyer, 1959). The remaining solids were repeatedly extracted in acetone until the supernatant lacked pigmentation. The lipid in the acetone solution was transferred to petroleum ether (PE) and along with the chloroform fraction from the Bligh and Dyer extraction was evaporated to dryness at 30 °C. Total lipid weight was determined gravimetrically after which the lipids were dissolved in benzene and stored at -20 °C until analysis. Total lipid weight of the nauplii was determined in a similar manner. Lipid weights are presented as mg lipid/g dry weight sample.

Fatty acids

The fatty acid composition of each sample was determined by gas-liquid chromatography on two independent columns as described by Schauer and Simpson (1978). Results are presented here as fatty acid methyl esters (FAME) weight percent of total lipid. Quantification of total FAME weights were made by co-injecting the FAME 20:2ω6 as an internal standard and are presented as mg FAME/g lipid of the dry weight samples.

Determination of energy content

Energy content of newly hatched nauplii was determined using wet oxidation in the presence of an acid-dichromate mixture (Maciolik, 1962). These values are reported as Joule/gram ash-free dry weight.

Data analysis

One-way analysis of variance was computed for total lipids, FAME and energy content. If significant differences ($P \leq 0.05$) were found, a Student-Neuman-Kuels posterior comparison was used to determine where the difference lay (Snedecor and Cochran, 1967).

Results

The Australian cysts' lipid level was statistically greater than the other four strains while the means of the Utah, SP 1628 and Brazilian strains were greater than that of the Italian cyst strain (Table I). The nauplii lipid levels, however, were all statistically different from one another except for the SF 321 and SP 1628 strains. The highest level of lipid in the nauplii was found in the Utah strain, and in descending order, the other strains were ranked as follows: Brazil, Australia, SF 313, SP 1628, SF 321 and Italy.

Table I

The amount of total lipid and fatty acid methyl esters in the cysts and newly hatched nauplii of five strains of *Artemia*[1]

	Artemia sources						
	Australia	Brazil	SF 313	SF 321	SP 1628	Italy	Utah
mg total lipids[2]							
Cysts	$157^{a} \pm 8$	$134^{b} \pm 8$	N.A.[3]	N.A.	$134^{b} \pm 22$	$91.0^{c} \pm 6$	$136^{b} \pm 1$
Nauplii	$185^{c} \pm 9$	$202^{b} \pm 8$	$174^{d} \pm 4$	$159^{e} \pm 13$	$160^{e} \pm 3$	$156^{f} + 2$	$224^{a} \pm 14$
mg fatty acid methyl esters[4]							
Cysts	$510^{b} \pm 18$	$502^{b} \pm 12$	N.A.	N.A.	$573^{b} \pm 39$	$704^{a} \pm 34$	$490^{b} \pm 19$
Nauplii	$751^{a,b} \pm 41$	$854^{a} \pm 26$	$716^{a,b} \pm 30$	$602^{b} \pm 15$	$711^{a} \pm 58$	$739^{a} \pm 12$	$742^{a,b} \pm 10$

[1] Values within each row which bear the same letter are not significantly different at $P \leq 0.05$.
[2] Per gram dry weight of sample.
[3] N.A. = not analyzed.
[4] Pe. gram lipid.

Although the total lipid level of the Italian strain was the lowest in both cysts and nauplii, it contained significantly greater levels of FAME (mg/g lipid) than the other statistically similar cyst strains. The FAME levels for the nauplii of the Brazil, Australia, Utah, Italy, SP 1628 and SF 313 strains were all statistically similar as were the Australian, Utah, SF 313 and SF 321 nauplii, but the Brazilian, Italian and SP 1628 strains were significantly greater than the SF 321 strain.

The fatty acid composition of cysts and nauplii is presented in Table II and Table III, respectively. The relative proportion of fatty acids in cysts and nauplii remain the same, indicating that the fatty acid content of the chorion is small.

TABLE II

Fatty acid composition of whole cysts of five strains of *Artemia*

FAME	Aust.	Brazil	SP 1628	Italy	Utah
14:0	1.80	2.04	0.65	1.79	1.20
14:1	2.11	1.03	2.88	3.55	1.94
15:0[1]	1.02	0.95	0.22	0.14	- - - -[1]
15:1	0.44	0.38	0.55	0.56	0.50
16:0	15.11	16.35	9.80	14.15	12.39
16:1	10.66	12.88	6.49	13.05	6.00
16:2ω7	0.58	0.68	1.67	2.04	1.68
16:3ω4/17:1ω8	3.98	3.93	2.69	3.47	1.47
18:0	2.66	2.34	2.38	3.31	3.55
18:1ω9	26.71	33.50	27.43	26.05	28.03
18:2ω6	6.22	9.17	5.30	7.08	5.58
18:3ω3	13 19	4.39	31.85	6.24	28.16
18:4ω3	4.41	0.97	5.15	1.55	3.52
20:1ω9	0.27	0.49	0.46	0.31	0.21
20:2ω6/ω9	0.08	0.29	0.15	0.62	0.16
20:3ω6	0.77	2.30	0.04	1.35	0.27
20:5ω3	9.32	8.35	1.66	12.61	3.23
22:6ω3	0.26	0.11	trace	- - - -[1]	trace

[1] No value (- - - -) means the fatty acid was not found.

The major fatty acids in all *Artemia* strains tested were 16:0, 16:1, and 18:1ω9. In addition, levels of 18:3ω3 and/or 20:5ω3 were substantial in various strains. Docosahexaenoic acid (22:6ω3) was found in only small amounts in the Australian and Brazilian *Artemia* nauplii. The *Artemia* strains were divided into two groups based on their most predominant long chain ω3 PUFA. Strains that contained mostly 18:3ω3 and 18:4ω3 included the SF 313, SP 1628, and Utah strains, while the group that contained predominantly 20:5ω3 included SF 321, Italian and Brazilian strains. The Australian strain contained substantial amounts of both 18:3ω3 and 20:5ω3 and therefore could be included in either of the two groups.

TABLE III

Fatty acid composition of newly hatched *Artemia*

FAME	Australia	Brazil	SF 313	SF 321	SP 1628	Italy	Utah
14:0	1.34	1.57	0.99	1.57	0.43	1.53	0.93
14:1	2.23	0.81	1.27	0.74	2.26	3.30	1.45
15:0	0.34	0.67	0.16	0.58	0.25	0.11	0 11
15:1	0.15	0.24	0.20	0.13	0.46	0.54	0.37
16:0	13.45	15.42	10.33	12.13	7.79	15.23	11.78
16:1	9.97	10.79	13.27	19.52	5.24	10.38	5.64
16:2ω7	- - - -[1]	- - - -[1]	- - - -[1]	- - - -[1]	1.51	2.94	- - - -[1]
16:3ω4/17:1ω8	3.87	3.88	2.09	2.32	2.44	3.28	2.90
18:0	3.07	2.79	6.83	2.90	3.08	3.17	4.07
18:1ω9	28.23	35.86	26.97	31.20	29.15	29.05	28.58
18:2ω6	5.78	9.59	9.35	3.69	4.60	6.79	4.60
18:3ω3	14.77	4.87	17.33	5.16	33.59	6.35	31.46
18:4ω3	4.37	0.96	3.26	1.28	4.88	1.01	3.10
20:1ω9	0.37	0.52	0.41	0.35	0.35	0.42	0.37
20:2ω6/ω9	0.12	0.06	0.06	- - - -[1]	0.24	0.20	0.09
20:3ω6	0.79	2.76	1.01	2.23	0.05	1.47	0.48
20:3ω3/20:4ω6	- - - -[1]	- - - -[1]	1.48	2.69	1.48	- - - -[1]	- - - -[1]
20:5ω3	10.50	8.98	4.06	12.44	1.68	13.63	3.55
22:6ω3	0.26	0.06	- - - -[1]	- - - -[1]	- - - -[1]	- - - -[1]	- - - -[1]

[1] No value (- - - -) means the fatty acid was not found.

Energy content for the geographical strains is presented in Table IV. Using the same statistical tests as in the lipid and FAME evaluations, differences in energy content between the various strains are significant ($P \leq 0.05$). The Australian strain contained the most energy with 2.50×10^4 J/g ash-free dry weight (5.961 Kcal/g); the Italian strain contained the least with 2.24×10^4 J/g ash-free dry weight (5.370 Kcal/g).

TABLE IV

The energy content[1,2] ($\times 10^4$) of five strains of *Artemia* nauplii

Australia	Brazil	SP 1628	Italy	Utah
$2.50^a \pm 0.16$	$2.35^{a,b} \pm 0.04$	$2.35^{a,b} \pm 0.11$	$2.24^b \pm 0.06$	$2.34^{a,b} \pm 0.08$

[1] J/g ash-free dry weight (ash content 5.4% dry weight).

[2] Values which bear the same letter subscript are not significantly different at $P \leq 0.05$.

Discussion

Major differences were found in the lipid level, FAME levels, fatty acid composition and the energy content of the cysts and nauplii of the five geographically different strains. These differences could have been caused by variations in the genetic make-up or the previous

dietary history of the parental stock which produced the cysts. Clark and Bowen (1976) have isolated six separate species from 27 different geographical strains of *Artemia* and Bowen *et al.* (1978) have shown a genetic variation in the hemolymph proteins of various strains. More recently, Abreu-Grobois and Beardmore (1980) found evidence for speciation between populations of *Artemia* and Seidel *et al.* (1980) showed a variation in the total protein electrophoretic patterns of the same five strains analyzed here.

The total FAME analysis provided an indication of the type of lipids associated with the various strains. A disproportionately high level of FAME for the Italian and a low value for the Utah strains indicated the two extremes. The Italian strain must have contained a higher level of triglyceride lipids per gram sample whereas the Utahan strain probably contains a greater proportion of phospholipids and/or sterol-type lipids.

The loss of the chorion, upon cyst hatching, resulted in a greater amount of lipid material per unit weight nauplii, except in the Italian strain. This would indicate that the chorion of the Italian strain contained more lipids than the shells of the other strains. The relative proportion of fatty acids of the cysts and nauplii remained the same before and after hatching and, when related to the increased lipid and FAME levels in the nauplii, indicates that the shell contains practically no fatty acids, with a possible exception of the Italian strain.

The nutritional quality of the five geographical strains used in this study has been recently determined for several species of marine larvae (Johns *et al.*, 1978, 1980 ; Beck *et al.*, 1980 ; Klein-MacPhee *et al.*, 1980). Johns *et al.* (1980), working with the larval stages of the mud crab *Rhithropanopeus harrisii* and the rock crab *Cancer irroratus* found marked differences in the ability of the geographical strains of brine shrimp nauplii to promote high survival and growth. Crab larvae did not complete larval development when fed SP 1628 or the Utah strain but did so when fed the Brazilian, Australian or Italian strains. Klein-MacPhee *et al.* (1980) found similar results when winter flounder *Pseudopleuronectes americanus* larvae were reared using the five geographical strains as food sources. Survival through metamorphosis was high in fish larvae fed Australian, Brazilian or Italian strains while it was markedly lower for larvae fed SP 1628 or Utah brine shrimp.

Beck *et al.* (1980), also working with the larvae of a marine fish (*Menidia menidia*), found varying results. Survival and growth of the fish larvae were dependent on several factors including the previous dietary history of the fish larvae. Differences in survival between the fish fed the various geographical strains were less prominent when all larvae were fed the Brazilian strain rather than the SP 1628 strain prior to the start of the experiment. In an earlier study, Johns *et al.* (1978) had found that both SF 313 and SF 321 promoted good survival throughout larval development of the mud crab. Beck *et al.* (1980) also showed that these two strains promoted higher (but not significant) survival in *Menidia menidia* than the SP 1628 strain.

The relatively good survival and growth of the marine organisms fed the Italian strain indicates that the lipid levels and energy content are probably adequate. The total energy values of the five strains were in close agreement with those of Paffenhöfer (1967) who found a level of 5 953 calories (2.49×10^4 J) per gram organic substance for an unidentified brine shrimp strain. Because the lipid levels and total energy values for the SP 1628 and Utah strains were greater, all strains were considered sufficient in energy and are probably not a significant contributor to the poorer growth and survival of the SP 1628 and Utah fed organisms.

The fatty acid composition of the nauplii appeared to be much more closely related to the biological effects of the five *Artemia* strains. The fatty acid composition of the strains evaluated here may have been different enough to affect their potential nutritional value. Strains that are higher in 20:5ω3 (Italy, SF 321, Brazil, and Australia) would presumably be better diets for marine organisms (Owen *et al.*, 1972 ; Yone and Fujii, 1975b ; Guary *et al.*, 1976 ; Watanabe *et al.*, 1978). Furthermore, the high level of 18:3ω3 (> 30% of the total FAME) and the low level of 20:5ω3 in the Utah and SP 1628 nauplii may have induced a nutritional stress in marine consumer organisms.

Watanabe *et al.* (1978) stated "that the class of EFA (essential fatty acids) contained in *Artemia* (principally 20:5ω3 and 22:6ω3) is the principle factor in the food value of *Artemia* to fish". This was determined by modifying the fatty acid composition of a San Francisco Bay Brand *Artemia* (1976), deficient in 20:5ω3 and 22:6ω3, by feeding them either a marine *Chlorella* or a yeast-supplemented squid liver oil, both of which are rich in these long chain ω3 PUFA. The enriched *Artemia* induced better growth and survival of their test organism, the red sea bream *Chrysophys major*.

Artemia have generally been shown to contain either a fatty acid predominance of 18:3ω3 or 20:5ω3 (Enzler *et al.*, 1974 ; Benijts *et al.*, 1976 ; Gallagher and Brown, 1975 ; Claus *et al.*, 1977, 1979 ; Watanabe *et al.*, 1978 ; Fujita *et al.*, 1980). Similar results were obtained in this study. Also, a variation was found in the fatty acid composition of cysts collected within the same year at a similar location (Table III, SF 313 *versus* SF 321) (personal communication, A. Schmidt, 1978).

One other important factor which relates to the lipid character of a food organism is the interaction of lipids with contaminants. This is important because a number of cyst strains are collected from salinas in locations near commercial and agricultural regions (*e.g.* San Francisco Bay, San Pablo Bay, and Great Salt Lake). An analysis of the five geographical strains for chlorinated hydrocarbons revealed that all are contaminated to some extent ; the Italian strain contained more of the DDT family of pesticides, the Utah and SP 1628 contained higher levels of dieldrin and the SP 1628 contained substantially higher levels of dieldrin and much higher chlordane levels than the other strains (Olney *et al.*, 1980).

The lipid content of a diet can affect the accumulation of pesticides which can thereby alter lipid metabolism. For example, Phillips and Buhler (1979) found that rainbow trout fed dieldrin-contaminated tubificid worms (15% lipid) experienced a decrease in lipid accumulation while fish fed an artificial diet (10% lipid) containing dieldrin exhibited normal rates of lipid accumulation. These authors suggested that the increased dietary lipid level created a greater reservoir for pesticide accumulation which, in turn, may have altered lipid metabolism. Furthermore, Durham (1967) has shown that dieldrin affects the metabolism of unsaturated fatty acids and accentuates the symptoms of a deficiency of essential fatty acids.

Considering the biological results of the mud crabs (Johns *et al.*, 1980) and winter flounder (Klein-MacPhee *et al.*, 1980) that were fed SP 1628 and Utahan strains of *Artemia*, it is possible that the excess 18:3ω3 and/or minimal quantities of 20:5ω3 could result in a nutritional stress which, in the presence of a dietary contaminant, could be manifested in a synergistic fashion. Both a nutritional stress and/or a pesticide contaminant could potentially exert a physiological response which may be elevated to a critical point at the time of metamorphosis. This may explain why the results of the five strains as diet on survival of Atlantic silversides (Beck *et al.*, 1980), which has a much less distinct metamorphosis, was not as

dramatic as in the mud crab and winter flounder. However, specific investigations will be required to evaluate these conditions.

Acknowledgements

This publication is a result of research sponsored by NOAA Office of Sea Grant, Department of Commerce, under Grant no. NA79AA-D-00096, and the University of Rhode Island Agricultural Experiment Station, contribution no. 1921.

The authors thank Mr Allan Beck and the marine culture team at the EPA – Environmental Research Laboratory – Narragansett for supplying the newly hatched nauplii.

Literature cited

Abreu-Grobois F. A. and J. A. Beardmore. 1980. International Study on *Artemia*. II. Genetic characterization of *Artemia* populations – an electrophoretic approach. p. 133-146. *In* : The brine shrimp *Artemia*. Vol. 1. Morphology, Genetics, Radiobiology, Toxicology. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 345 p.

Beck A. D., D. A. Bengtson, and W. H. Howell. 1980. International Study on *Artemia*. V. Nutritional value of five geographical strains of *Artemia* : effects on survival and growth of larval Atlantic silverside *Menidia menidia*. p. 249-259. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

Benijts F., E. Vanvoorden, and P. Sorgeloos. 1976. Changes in the biochemical composition of the early larval stages of the brine shrimp, *Artemia salina* L. p. 1-9. *In* : Proc. 10th European Symp. Marine Biology. Vol. 1. Mariculture. Persoone G. and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 620 p.

Bligh E. G. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911-917.

Bowen S. T., J. P. Durkin, G. Sterling, and L. S. Clark. 1978. *Artemia* hemoglobins : genetic variation in parthenogenetic and zygogenetic populations. *Biol. Bull.* 155:273-287.

Castell J. D. and J. F. Covey. 1976. Dietary lipid requirements of adult lobsters (*Homarus americanus* M.E.). *J. Nutr.* 106:1159-1165.

Castell J. D., D. J. Lee, and R. O. Sinnhuber. 1972a. Essential fatty acids in the diet of rainb[illegible]out (*Salmo gairdneri*) : lipid metabolism and fatty acid composition. *J. Nutr.* 102:93-99.

Castell J. D., R. O. Sinnhuber, J. H. Wales, and D. J. Lee. 1972b. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*) : growth, feed conversion and some gross deficiency symptoms. *J. Nutr.* 102:77-86.

Clark L. S. and S. T. Bowen. 1976. The genetics of *Artemia salina*. VII. Reproductive isolation. *J. Hered.* 67: 385-388.

Claus C., F. Benijts, and P. Sorgeloos. 1977. Comparative study of different geographic strains of the brine shrimp *Artemia salina*. p. 91-105. *In* : Fundamental and applied research on the brine shrimp *Artemia salina* (L.) in Belgium. Persoone G. and E. Jaspers (Eds). European Mariculture Society Special Publication No. 2. EMS, Bredene, Belgium. 110 p.

Claus C., F. Benijts, G. Vandeputte, and W. Gardner. 1979. The biochemical composition of the larvae of two strains of *Artemia salina* (L.) reared on two different algal foods. *J. exp. mar. Biol. Ecol.* 36:171-183.

Cowey C. B., J. M. Owen, J. W. Adron, and C. Middleton. 1976. Studies on the nutrition of marine flatfish. The effect of different dietary fatty acids on the growth and fatty acid composition of turbot (*Scophthalmus maximus*). *Br. J. Nutr.* 36:479-486.

Durham W. F. 1967. The interaction of pesticides with other factors. *Residue Review* 18:21-103.

Enzler L., V. Smith, J. S. Linn, and H. S. Olcott. 1974. The lipids of Mono Lake brine shrimp (*Artemia salina*). *J. Agr. Food Chem.* 22:330-331.

Fujita S., T. Watanabe, and C. Kitajima. 1980. Nutritional quality of *Artemia* from different localities as a living feed for marine fish from the viewpoint of essential fatty acids. p. 277-290. *In* : The brine shrimp *Artemia*. Vol. 3.

Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

GALLAGHER M. and W. D. BROWN. 1975. Composition of San Francisco Bay brine shrimp (*Artemia salina*). *J. Agr. Food Chem.* 23:630-632.

GUARY J. C., M. KAYAMA, Y. MURAKAMI, and H. J. CECCALDI. 1976. The effect of fat-free and compounded diets supplemented with various oils on moult, growth and fatty acid composition of prawn, *Penaeus japonicus* Bate. *Aquaculture* 7:245 254.

JOHNS D. M., M. E. PETERS, and A. D. BECK. 1978. International Study on *Artemia*. I. An evaluation of five geographical strains of brine shrimp, *Artemia salina*, as food for larval mud crabs, *Rhithropanopeus harrisii*. *Amer. Zool.* 18:585. (abstract).

JOHNS D. M., M. E. PETERS, and A. D. BECK. 1980. International Study on *Artemia*. VI. Nutritional value of geographical and temporal strains of *Artemia* : effects on survival and growth of two species of brachyuran larvae. p. 291-304. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

KANAZAWA A., S. TESHIMA, S. TOKIWA, and H. J. CECCALDI. 1979. Effects of dietary linoleic and linolenic acids on growth of prawn. *Oceanol. Acta* 2(1):41-47.

KLEIN-MACPHEE G., W. H. HOWELL, and A. D. BECK. 1980. International Study on *Artemia*. VII. Nutritional value of five geographical strains of *Artemia* to winter flounder *Pseudopleuronectes americanus* larvae. p. 305-312. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

MACIOLIK J. 1962. Limnological organic analysis by quantitative dichromate oxidation. *Fish. Res. Report* 60:1-61.

NORRED W. P. and A. E. WADE. 1972. Dietary fatty acid-induced alterations of hepatic microsomal drug metabolism. *Biochem. Pharm.* 21:2887-2897.

OLNEY C. E., P. S. SCHAUER, S. MCLEAN, You LU, and K. L. SIMPSON. 1980. International Study on *Artemia*. VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory-reared marine fish. p. 343-352. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

OWEN J. M., J. W. ADRON, J. R. SARGENT, and C. B. COWEY. 1972. Studies on the nutrition of marine flatfish. The effect of dietary fatty acids on the tissue fatty acids of the plaice *Pleuronectes platessa*. *Mar. Biol.* 13:160-166.

PAFFENHÖFER G. A. 1967. Caloric content of larvae of the brine shrimp *Artemia salina*. *Helgoländer wiss. Meersunters.* 16:130-135.

PANDIAN T., 1975. Mechanisms of heterotrophy. p. 61-250. *In* : Marine ecology. Vol. 2, Part 1. Kinne O. (Ed.). J. Wiley and Sons, New York. 449 p.

PHILLIPS G. R. and D. R. BUHLER. 1979. Influences of dieldrin on the growth and body composition of fingerling rainbow trout (*Salmo gairdneri*) fed Oregon Moist Pellet or tubificid worms (*Tubifex sp.*). *J. Fish. Res. Bd Can.* 36:77-80.

SCHAUER P. S. and K. L. SIMPSON. 1978. Effects of diets on the fatty acid composition of juvenile Atlantic silversides (*Menidia menidia*). p. 175-187. *In* : Proc. 9th Annual Meeting World Mariculture Society. Avault J. W., Jr. (Ed.). Louisiana State University, Baton Rouge, Louisiana. 807 p.

SEIDEL C. R., J. KRYZNOWEK and K. L. SIMPSON. 1980. International Study on *Artemia*. XI. Amino acid composition and electrophoretic protein patterns of *Artemia* from five geographical locations. p. 375-382. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

SNEDECOR G. W. and W. G. COCHRAN. 1967. Statistical methods. Iowa State Univ. Press, Ames, Iowa. 593 p.

WATANABE T., F. OAWA, C. KITAJIMA, and S. FUJITA. 1978. Nutritional quality of brine shrimp, *Artemia salina*, as a living feed from the viewpoint of essential fatty acids for fish. *Bull. Jap. Soc. Sci. Fish.* 44:1115-1121.

YONE Y. and M. FUJII. 1975a. Studies on nutrition of red sea bream. XI. Effect of $\omega 3$ fatty acid supplement in a corn oil diet on growth rate and feed efficiency. *Bull. Jap. Soc. Sci. Fish.* 41:37-77.

YONE Y. and M. FUJII. 1975b. Studies on nutrition of red sea bram. XII. Effect of $\omega 3$ fatty acid supplement in a corn oil diet on fatty acid composition of fish. *Bull. Jap. Soc. Sci. Fish.* 41:79-86.